UNIVERZITET U KRAGUJEVCU
AGRONOMSKI FAKULTET U ČAČKU

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FACULTY OF AGRONOMY
CACAK

XXIV SAVETOVANJE O BIOTEHNOLOGIJI

sa međunarodnim učešćem

- ZBORNIK RADOVA 1 -

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-Zbornik radova 1-

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PREDGOVOR

Promene koje se ubrzano dešavaju na globalnom i lokalnom nivou od naučnih, klimatskih, ekonomskih pa do političkih podstiču potrebu da proučimo njihov uticaj na živi svet i na jednu od najvažnijih ljudskih delatnosti - proizvodnju hrane.

Naša poljoprivreda, naše selo, naši poljoprivredni proizvođači nisu danas ono što su i pre trideset, četrdeset ili manje godina bili, srpsko selo se danas više nego ikad ubrzano i u hodu menja. Poljoprivredna nauka mora preuzeti deo odgovornosti u pogledu proizvodnje dovoljne količine kvalitetne hrane za ljudsku ishranu jer prolaze vremena kada se za svaku lošu žetvu traže opravdanja u klimi.

Sa ciljem da budemo u toku određenih zbivanja, kao i da sami svojim rezultatima utičemo na razvoj poljoprivrede i njenih pratećih delatnosti osim kroz edukaciju studenata, Agronomski fakultet u Čačku organizuje i Savetovanje o biotehnologiji.

Osnovni cilj Savetovanja je upoznavanje šire naučne i stručne javnosti sa rezultatima najnovijih naučnih istraživanja, domaćih i inostranih naučnika iz oblasti osnovne poljoprivredne proizvodnje i prerade i zaštite životne sredine. Na taj način fakultet nastoji da omogući direktan prenos naučnih rezultata široj proizvodnoj praksi, pa pored naučnih radnika, agronoma, tehnologa, na ovogodišnjem Savetovanju biće i značajan broj poljoprivrednih proizvođača, stručnih savetodavaca, nastavnika, itd.

U Zborniku radova XXIV Savetovanja o botehnologiji sa međunarodnim učešćem, predstavljeno je ukupno 126 radova iz oblasti Ratarstva, Povrtarstva i Krmnog bilja, Voćarstva i vinogradarstva, Zootehnike, Zaštite bilja, proizvoda i životne sredine i Prehrambene tehnologije.

Pokrovitelj za XXIV Savetovanje o biotehnologiji sa međunarodnim učešćem je Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije, a materijalnu i organizacionu podršku su nam pružili grad Čačak, privrednici, dugogodišnji prijatelji Agronomskog fakulteta, kojima se i ovim putem zahvaljujemo.

Kolektivu Agronomskog fakulteta, takođe dugujemo zahvalnost, jer su i ovaj put radnici svih struktura, svako na svoj način, doprineli realizaciji još jednog Savetovanja.

U Čačku, marta 2019. godine

Programski i Organizacioni odbor
XXIV Savetovanja o biotehnologiji

SADRŽAJ

Sekcija: Ratarstvo, povrtarstvo i krmno bilje

EFFECT OF EXTRACTION SOLVENTS ON THE ANTIOXIDANT ACTIVITY OF INDUSTRIAL HEMP EXTRACTS

Dejan Prvulović[1], Sonja Gvozdenac[2], Marijana Peić Tukuljac[1], Đorđe Malenčić[1], Biljana Kiprovski[2], Vladimir Sikora[2], Dragana Latković[1]

Abstract: The objective of this study was to determine the content of phenolic compounds (total phenolics, total tannins and total flavonoids) and antioxidant capacity by six different assays in industrial hemp ('Helena' variety) extracted by four different extraction solvents: 70% methanol, 70% ethanol, 70% acetone and water. Out of the four solvent mixtures evaluated in the current study for the extraction of phenolic compounds, the use of 70% acetone yielded to the highest total contents of phenolics and exhibited the highest antioxidant activity in all performed assays.

Key words: antioxidant capacity, *Cannabis sativa* L., hemp, phenolics

Introduction

Industrial hemp (*Cannabis sativa* L.) is a quick growing annual dioecious or monecious plant with a multitude of uses. Morphologically, the male and the female plants do not differ much with regards to their vegetative growth and they can be identified only during flowering (Kaushal, 2012).

Hemp is known throughout the world and has been cultivated for thousands of years. Because of its unique properties, hemp is valuable for the bio-based economy. Hemp can be grown as a fiber, seed, or dual-purpose crop. Hemp fibers are used in fabrics and textiles, paper, construction and insulation materials etc. However, hemp has far more potential as an oilseed crop than as a fiber crop at this time (Karus and Vogt, 2004). Industrial hemp is currently witnessing a revival, because of its rich spectrum of bioactive compounds, its fibers and its agricultural features, namely lower water requirement with respect to other crops, good resistance to pest and drought, well-developed root system preventing soil erosion etc. (Andre et al., 2016).

There is also a growing interest over the valorization of hemp secondary metabolites. Seeds of hemp are a rich source of proteins, arginine and essentially polyunsaturated fatty acids. The whole plant is rich in different phytochemicals, including cannabinoids, terpenes and phenolic compounds (Andre et al., 2016). Flours from hemp are also a rich source of bioactive compounds from the polyphenols group (Mikulec et al., 2019).

The aim of this study was to investigate the antioxidant capacity of industrial hemp cv. 'Helena' affected by different extraction solvents and to determine the relationship between the antioxidant activity of extracts and different phenolic groups in hemp flowering tops.

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Material and methods

The analysis was performed on industrial (fiber) monoecious hemp variety 'Helena' supplied by the Institute of Field and Vegetable Crops, Department for Alternative Crops, Novi Sad, Serbia. Plants used for analysis were harvested in flowering phase and only flowering tops were used. Industrial hemp flowering tops were dried till constant weight and then grounded to a fine powder using laboratory mill. One gram of plant material was extracted overnight with 50 mL of 70% acetone, 70% ethanol or 70% methanol. Aqueous extracts were prepared with boiling distillated water. The extracts were filtered and kept refrigerated until assayed.

The contents of total phenolics (TP) and total tannins (TT) was determined using a Folin-Ciocalteu colorimetric method (Nagavani and Raghava Rao, 2010) and the results were expressed in milligrams of quercetin equivalents per 1 g of dry plant weight (mg QE/g). Data are reported as means for at least three replications for all performed assays. The total flavonoids (TF) content was determined spectrophotometrically (Saha et al., 2013). The amount of flavonoids was calculated as a quercetin equivalent (QE) from the calibration curve of quercetin standard solutions.

Scavenging of free radicals was tested in a DPPH (2,2-diphenyl-1-picrylhydrazyl) acetone solution (Lai and Lim, 2011). The degree of decoloration of solution indicates the scavenging efficiency of the substance added. The standard curve was constructed using different concentrations of Trolox, and the results were expressed as mg Trolox equivalents per gram of dry plant material (mg TE/g). Ferric-reducing antioxidant power (FRAP) assay was carried out according to the procedure described in the literature (Valentão et al., 2002). The ABTS assay was based on a method developed by Miller et al. (1993). Methanolic solution of known Trolox concentrations were used for calibration and the results were expressed as mg Trolox equivalents per g of dry plant material (mg TE/g). The total antioxidant activity (TAA) of plant extracts were evaluated by phosphomolybdenum method as reported by Kalaskar and Surana (2014). Trolox was used as a standard. A reducing power assay (total reduction capacity-TRC) was performed by method of Saha et al. (2013). The standard curve for total antioxidant activity was plotted using Trolox solution. The superoxide free radical scavenging activity was carried out by NBT (nitroblue tetrazolium) test (Kalaskar and Surana, 2014). The results were expressed as number of International Units (IU) of superoxide dismutase (SOD) equivalents per gram of dry plant material (IU SOD/g).

Statistical analysis. Results were expressed as a mean value of determinations of 3 independent samples made in triplicates. Statistical significance was tested by analysis of variance followed by a comparison of means by Duncan's multiple range test ($P < 0.05$) calculated using STATISTICA for Windows version 12.0 (StatSoft, Tulsa, OK, USA). Stepwise multiple regression analyses were used to determine correlation among variables.

Results and discussion

The content of total phenolics (TP), total tannins (TT) and total flavonoid (TF) was examined and the results are presented in table 1. Content of phenolic compounds

depends on many different factors: The different solvent extraction systems showed a wide range of TP concentrations from 2.107 up to 6.316 mg QE/g. The TP content values were significantly different among different solvent systems used for extraction. In particular, 70% acetone extract of flowering tops showed the highest TP content, followed by 70% ethanol and 70% methanol extracts while the aqueous extract possesses the lowest concentration of TP. This data are partially in the agreement with results of other researchers (Mkpenie et al., 2012). Our previous research on soybean seeds demonstrates that 70% acetone solvent is superior solvent over 70% methanol and 70% ethanol systems in extracting TP from plant material (Prvulović et al., 2016). Kosakowska et al. (2018) also found that ethanolic (60%) solvent system extract significantly more phenolic components compared with pure water extraction system from roseroot underground organs.

Tabela 1. Sadržaj fenolnih jedinjenja u ekstraktima konoplje
Table 1. Content of phenolic compounds in extracts of industrial hemp

	Estrakcioni rastvarač *Extraction solvent*			
Parametar *Parameter*	Voda *Water*	70% metanol *70% Methanol*	70% etanol *70% Ethanol*	70% aceton *70% Acetone*
Ukupni fenoli[1] *Total phenolics[1]*	2.107 ± 0.301a	5.576 ± 0.063b	5.767 ± 0.260b	6.316 ± 0.224c
Ukupni tanini[1] *Total tannins[1]*	0.760 ± 0.036a	2.378 ± 0.148b	2.321 ± 0.113b	0.929 ± 0.025c
Ukupni flavonoidi[1] *Total flavonoids[1]*	0.125 ± 0.014a	3.740 ± 0.167b	4.467 ± 0.026c	3.245 ± 0.689b

[1]mg kvercetina/g suvog biljnog materijala
[1]mg quercetin equivalents/g dry plant material (mg QE/g)
a-c vrednosti u okviru istog reda sa različitom slovnom oznakom se statistički značajno razlikuju ($P < 0,01$)
a-c values without the same letter within each row differ significantly ($P < 0.01$)

Tannins are very useful from an agricultural point of view, as a protector from biotic stress, but they are undesirable compounds when plant-parts are consumed as food. Tannins are synthesized in plants not only through genetic determinants, physiological demands and evolution-controlled protection needs, but also by the influence of biotic and abiotic stress factors (Furlan et al., 2010). The range of total tannins in tested hemp flowering tips varied between 0.760 and 2.378 mg QE/g. Methanolic and ethanolic solvent systems extracted 2-3 times more total tannins from hemp than aqueous and acetone extraction systems.

Flavonoids are low molecular weight polyphenolic secondary metabolic molecules and play a variety of significant roles in plants. Flavonoids act as detoxifying agents,

signal molecules, stimulants for germination, stress resistance, phytoalexines etc (Samanta et al., 2011). However, a high intrapopulation variability concerning the content and composition of flavonoids were found in different plant species (Kosakowska, 2017). The total flavonoids content in flowering tips of industrial hemp ranged from 0.125 mg QE/g (aqueous extract) to 4.467 mg QE/g (70% ethanol extract).

Tabela 2. Antioksidativna aktivnost ekstrakata konoplje
Table 2. Antioxidant activity of extracts of industrial hemp

	Estrakcioni rastvarač *Extraction solvent*			
Test *Test*	Voda *Water*	70% metanol *70% Methanol*	70% etanol *70% Ethanol*	70% aceton *70% Acetone*
DPPH[1,3] *DPPH[1,3]*	1.740 ± 0.105a	3.433 ± 0.082b	3.600 ± 0.348b	5.517 ± 0.303c
FRAP[1,4] *FRAP[1,4]*	9.470 ± 0.794a	9.016 ± 0.464a	9.996 ± 0.229a	11.673 ± 0.398b
ABTS[1,5] *ABTS[1,5]*	26.148 ± 2.899a	45.758 ± 3.634b	51.633 ± 3.071b	50.108 ± 2.440b
NBT[2,6] *NBT[2,6]*	0.674 ± 0.047a	0.714 ± 0.074a	1.194 ± 0.007b	1.427 ± 0.147c
URK[1,7] *TRC[1,7]*	3.472 ± 0.191a	7.561 ± 0.406b	10.055 ± 0.726c	9.300 ± 0.287c
UAA[1,8] *TAA[1,8]*	60.88 ± 5.90a	97.10 ± 8.47b	115.00 ± 12.42c	143.08 ± 11.39d

[1]mg troloksa/g suvog biljnog materijala; [1]*mg trolox equivalents/g dry plant material*
[2]IU SOD/g suvog biljnog materijala; [2]*IU SOD equivalents/g dry plant material*
[3]*DPPH-2,2-diphenly-1-pycrylhydrazyl*
[4]*FRAP-Ferric-reducing antioxidant power*
[5]*ABTS-2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)*
[6]*NBT-Nitroblue tetrazolium*
[7]URK-Ukupni redukcioni kapacitet; [7]*TRC-Total Reduction Capacity*
[8]UAA-Ukupna antioksidativna aktivnost; [8]*TAA-Total Antioxidant Activity*
a-c vrednosti u okviru istog reda sa različitom slovnom oznakom se statistički značajno razlikuju ($P < 0,01$)
a-c values without the same letter within each row differ significantly ($P < 0.01$)

An antioxidant is a chemical compound that prevents the oxidation of substrates. They protect the key cell compounds and components by neutralizing the damaging effects of different free radicals. The different types of assays and methods published in the scientific literature for the determinations of antioxidant activity of different biological systems involve more than one mechanism. There must be used more than one method for comparing the mode of antioxidant action of selected compound or mixture (Shalaby and Shanab, 2013). Antioxidant capacities of different extracts of flowering tips of industrial hemp were determined with six different assays. Antioxidant activities measured in four different extracts obtained using FRAP, ABTS, DPPH, NBT, TAA and TRC assays are presented in Table 2. The results obtained in this work showed that the hemp acetone extract possesses the strongest antioxidant activity, followed by ethanolic and methanolic

extracts, compared to the aqueous extract. Presented results are mostly in agreement with work of other researchers (Kosakowska et al., 2018; Meneses et al., 2013; Stroe et al., 2018; Zhang, 2015) on different plant species.

Conclusion

The results of the present investigation revealed that phenolic compound contents and antioxidant capacity of extracts of industrial hemp are significantly affected by the solvent system used for the extraction process. Out of the four solvent mixtures evaluated in the current study for the extraction of phenolic compounds, the use of 70% acetone yielded to the highest total contents of phenolics and exhibited the highest antioxidant activity in most of the assays applied.

The use of pure distilled water as the solvent resulted in lowest extraction of all measured phenolic compounds and lowest antioxidant capacity. Data on phenolic compounds investigated in this study, as well as the antioxidant activity of extracts of industrial hemp could be valuable to the food and pharmaceutical industries.

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